IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Adachi, et al.
Serial No.:	10/517,804
For:	FLAVOR DETERIORATION INHIBITOR AND INHIBITOR FOR THE GENERATION OF CITRAL DETERIORATION SMELL
Filed:	December 10, 2004
Examiner:	Nikki H. Dees
Art Unit:	1794
Confirmation No.:	8937
Customer No.:	27,623 Docket No.: 3019.010USU

DECLARATION UNDER 37 CFR 1.132

I, Susumu KIYOHARA, declare and state:

1. I am a co-inventor of the invention as claimed in the subject patent application.

2. I graduated from the master's degree program in Materials Science, Graduate School of Engineering of Tottori University.

3. I entered employment with Ogawa & Co., Ltd. in 1998, and am currently the Researcher in the Material R & D Laboratory.

I am now engaged in the research and development business of flavor and fragrance materials.

I have more than 11 years of technical experience and formal education in Flavor and Food Chemistry.

4. I have read and understood the contents as the Examiner pointed out for the subject patent application in view of Bank et al. (WO 98/58656).

5. The following experiment was carried out under my own instructions.

5.1 Experiment

1) Object of Experiment

The object was to evaluate comparatively anti-oxidant activities of the "black tea extract" prepared by Extraction Example 24 in the subject patent application and

rosmarinic acid which is disclosed in Bank et al.

2) Procedures

i) Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), citric acid (anhydrous) and L-ascorbic acid used in this experiment were purchased from Nakarai Tesque, Inc., rosmarinic acid from Funakoshi Co., Ltd., and disodium hydrogen phosphate from HIROSHIMA WAKO Pure Chemical Industries, Ltd.

The black tea extract was prepared according to the Extraction Example 24 in the subject patent application as under-mentioned.

-- Extraction was carried out by adding 500 g of a 50 % by weight aqueous solution of ethanol to 50 g of black tea leaves and heating under reflux for one hour. After removing insoluble by filtration, the filtrate was concentrated under reduced pressure and freeze-dried to give 15.1 g of a brown powder. --

ii) Measurement of DPPH radical scavenging activity

① Preparation of DPPH-ethanol solution

DPPH was precisely weighed (0.01856 g), 99.5 % ethanol was added to make up 200 ml for complete dissolution.

② Preparation of citric acid・disodium hydrogen phosphate buffer (pH 5.5)

Citric acid (anhydrous) was precisely weighed (3.842 g), and 50 % ethanol (w/w) was added to make up 200 ml. Disodium hydrogen phosphate (dodecahydrate) was precisely weighed (35.814 g) and 50 % ethanol was added to make up 500 ml. These solutions were used to prepare a citric acid・disodium hydrogen phosphate buffer (pH 5.5).

③ Preparation of samples

L-ascorbic acid was precisely weighed (0.05 g) and 50 % ethanol was added to make up 100 ml. A 0.5 ml portion of this solution was weighed out and 50 % ethanol was added to make up 50 ml. And further, the same dilution procedure was repeated 4 times and prepared for different concentration samples. Rosmarinic acid was subjected to the same procedures to prepare samples.

On the other hand, the black tea extract was precisely weighed (0.05 g) and 50 % ethanol was added to make up 100 ml. A 1 ml portion of the resulting solution was weighed out and 60 % ethanol was added to make up 50 ml. Then, a 0.5 ml portion of this solution was weighed out and 50 % ethanol was added to make up 10 ml. And further, the same

procedures were repeated to perform dilution up to four stages.

④ Measurement

The resultant preparations were used as samples. 2 ml of each sample, 2 ml of the citric acid・disodium hydrogen phosphate buffer (pH 5.5) and 1 ml of the DPPH-ethanol solution were successively added. After stirred well, the resulting mixture was allowed to stand at room temperature in the dark place for 30 minutes. Then, absorbance at 512 nm was determined using a spectrophotometer (manufactured by Shimazu Corp.: UV-2450) (using silica glass, 1 cm cell). A sample, to which 99.5 % ethanol was added instead of the sample solution, was used as a control for absorbance.

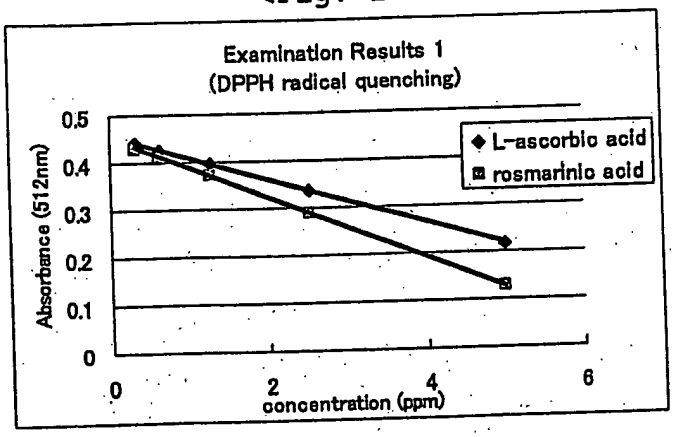
⑤ Evaluation

Evaluation of anti-oxidant activity was made by measuring the DPPH radical scavenger activity for each sample and determining a relative value to the DPPH radical scavenging activity of L-ascorbic acid.

3) Results

Fig. 1 shows the relationship between concentration of each sample and absorbance thereof. Approximate curve was drawn from these plotted points, absorbance (Abs) of the control was defined as 100 % and a sample concentration to yield 50 % (Abs/2) (IC_{50}) was determined. If the IC_{50} value of a substance (A) is lower than that of a substance (B), it means that the substance (A) has higher antioxidative activity than the substance (B). So, the antioxidative activity of each sample was evaluated by the IC_{50} value measured in this way.

<Fig. 1>



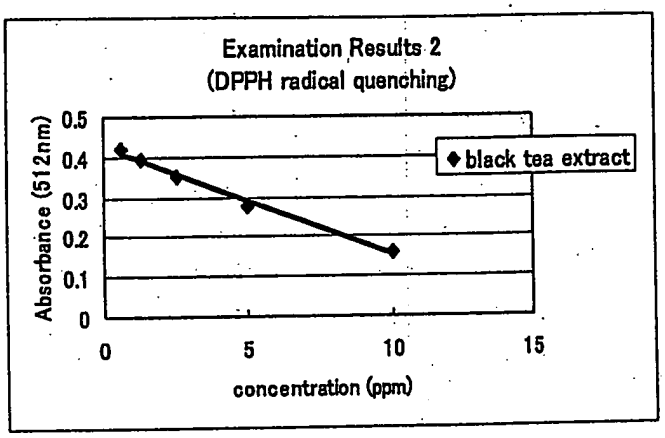
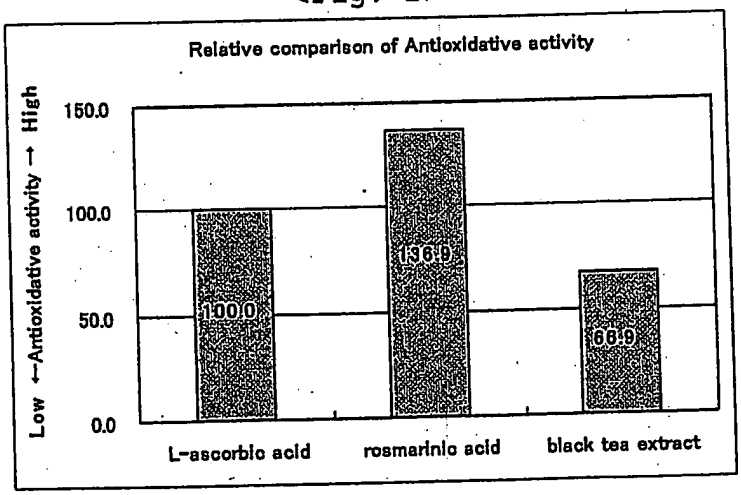


Fig. 2 shows the results obtained from relative comparison of the reciprocal of the IC_{50} for the respective samples. Rosmarinic acid exhibited very higher activity as compared with L-ascorbic acid, whereas the black tea extract exhibited very lower activity as compared with L-ascorbic acid. It could have thus been judged that rosmarinic acid exerts a higher radical scavenging activity than the black tea extract.

<Fig. 2>



5.2 Conclusion

As a result from the DPPH radical scavenger activity test, it has been proven that the anti-oxidant activity of the black tea extract was lower as compared to rosmarinic acid.

Based on my analysis of this experimental test data, my review of Bank et al., as well as my experience and education in the technology, my opinion is that it would not have been obvious for one of ordinary skill in the art to have substituted an extract from tea for the rosemary

extract of Bank et al.

6. I further declare that all statements made herein are true and that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: February 26, 2010

By Susumu Kiyohara
Susumu KIYOHARA